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**Merabti-Brahim**
A) Department of Biology, Faculty of Science of the Nature and the Life, Amar Telidji University, Laghouat, Algeria
B) Team desertification and climate, Mechanics Laboratory, Amar Telidji University, Laghouat, Algeria
C) Applied Neuro-endocrinology Laboratory. Department of Biology, BP 12, Faculty of Sciences, University of Badji Mokhtar-Annaba., Algeria

**Lebouz-Ismahan**
Department of Biology, Faculty of Science of the Nature and the Life, Mohamed Kheider University, Biskra, Algeria

**Adamou-Ala Eddine**
Team Desertification and Climate, Mechanics Laboratory, Amar Telidji University, Laghouat, Algeria

**Ouakid-Mohamed Laid**
Applied Neuro-Endocrinology Laboratory. Department of Biology, BP 12, Faculty of Sciences, University of Badji Mokhtar-Annaba., Algeria

**Correspondence**
**Merabti-Brahim**
A) Department of Biology, Faculty of Science of the Nature and the Life, Amar Telidji University, Laghouat, Algeria
B) Team desertification and climate, Mechanics Laboratory, Amar Telidji University, Laghouat, Algeria
C) Applied Neuro-endocrinology Laboratory. Department of Biology, BP 12, Faculty of Sciences, University of Badji Mokhtar-Annaba., Algeria

# The reproductive potential of two species of *Culicidae* treated with fruit extract of *Citrullus colocynthis* (L.) Schrad 1838

**Merabti-Brahim, Lebouz-Ismahan, Adamou-Ala Eddine and Ouakid-Mohamed Laid**

**Abstract**
The preliminary trials of the aqueous extract of *Citrullus colocynthis* fruits on the larvae ($L^4$) of two species of mosquitoes (*Culex pipiens* and *Culiseta longiareolata*) showed the efficacy of this extract on the larvae (L4) of the two species. The sublethal doses (10, 50 and 100 mg / l) were tried on the reproductive performances to the two species studied. The results showed that there was a reduction in female fertility of the emerged larvae exposed to these doses. For *Culex pipiens*, the number of eggs laid was 409, 261 and 123 eggs respectively, with the doses 10, 50 and 100 mg / l, while at *Culiseta longiareolata*, pundits were 431, 302 and 150 eggs. Knowing that in the control females, the rate of fertility was high compared to treated individuals; the number of eggs laid was 598 and 612 respectively for the first and second species.

**Keywords:** *Citrullus colocynthis, Culex pipiens, Culiseta longiareolata*, reproductive, fertility

## 1. Introduction
Recently, the Sahara of Algeria experienced a fatal spread of the *Culicidae* fauna [1-6]. The study of larval habitats, behavior and ecology of these vectors is a good way to fight against them [7-8]. The emergence of insect resistance against synthetic insecticides such as *Bacillus thuringiensis*, *Bacillus sphaericus*, for example, has encouraged scientists to find alternatives and strategies, including the use of natural substances, which have Action in pharmacology, such as bactericides, fungicides, acaricides [9-11]. The use of plant extracts as insecticides has been known for a long time. Indeed pyrethrum, nicotine and rotenone are already known as control agents against insects. There are over 2000 plant species which are known to have insecticidal activity are already identified [11-12]. Recently, the use of new insect control methods, such as reduction of fertility in females or sterility in males, has been supported by several researchers [13-16]. Our attention through this study is intended to find ways to effectively fight against insects. For this reason, we attempted to try the toxic effect of the aqueous extract fruits of *Colocynthis citrullus* against the larval stage ($L^4$) of the two species of mosquitoes (*Culex pipiens* and *Culiseta longiareolata*). The effect of sublethal doses on the reproductive performance of these two species, considered as objective in this study.

## 2. Materials and Methods
### 2.1 Presentation of the plant and the preparation of the aqueous extract
*Citrullus colocynthis* is an Annual grass with climbing stem; the fruit is spherical and smooth, first of a spotted green, then yellowish when ripe. This plant originates from the hottest parts of Asia and Africa [17]. Plant material was collected from the Laghouat region (33° 48′ 24″ N,2° 52′ 56″ E) (Algeria) in March 2014; fruits are beforehand washed in the distilled water then dried in a steam room carried in 40 °C during 48 to 72 hour. It is then crushed to powder. A quantity of 100 g of powder of plant is diluted in a liter of water distilled beforehand carried in boiling, then let cool under magnetic excitement counterparts 30 minutes. The obtained mixture is filtered by the Whatman paper (3 mm). The filtrate obtained has a stock solution of 10%.

### 2.2 Larval sampling and breeding

The larvae of *Cx. pipiens* and *Cs. longiareolata* were collected from the Lak of Minea (Ghardaia; Algeria 30 ° 35′ 20″ N 2 ° 52′ 47″ E) Twice a month in 2014. The larvae were reared in storage jars containing 500 ml of stored tap water and maintained at a temperature from 25-30 °C, 85% RH and a photoperiod of 14:10 (L:D).Larvae were fed daily with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight), and water was changed every four days [18]. The feeding continued until the larvae were transformed into the pupa stage. The pupae were transferred from the trays to a cup containing tap water and placed in screened cages (30×30×30cm) where the adults emerged. After emergence, female mosquitoes obtained blood meal from caged pigeons, while male mosquitoes were fed a 10% sucrose solution. Then, egg masses were kept to continue the next generation.

### 2.3 The attempts in the laboratory and the statistical analysis

After preliminary tests to determine Lethal doses, the effect of the extract used with four doses (10, 50, 100 and 200 mg / L) on the mosquitoes larva for the toxic behavior. The adults appeared from every group (witness and treated) for the first three doses are placed in wages separately to obtain eggs after the coupling up of Male and females. Females filled with some blood are placed in cages packaging of prizes filled with water distilled for the laying. We calculate the parameters of reproduction of every group according to the number of laid, hatched and appeared eggs [19]. The statistical tests for the parameters of reproduction are calculated with Statistix v8.0.

$$\text{The percentage of hatching} = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} X\ 100$$

$$\text{Fecunidity} = \frac{\text{Total number of hatched eggs}}{\text{Total number of females}} X\ 100$$

## 3. Results

### 3.1 Effect of *Citrullus colocynthis* on the fertility of females treated

The treatment of the larva of the fourth stage by three doses 10, 50 and 100 mg/l, reduced the fecundity and the fertility of females treated. The table 1 indicated a significant difference between eggs laid by the treated and witness for both species *Cx. pipiens* and *Cs. longiareolata* ($p \leq 0, 0001$).

**Table 1:** number of the eggs laid by treated females of *Cx. pipiens* and *Cs. longiareolata*

| Species | doses (mg/L) | Mean ± Sem | Min | Max | F | P (0,05) |
|---|---|---|---|---|---|---|
| *Culex pipiens* | Witness | 59,80±4,34 | 54 | 68 | $F^{3, 39}$=185 | .000 |
| | 10 | 40,9±1,91 | 38 | 44 | | |
| | 50 | 26,1±4,09 | 20 | 31 | | |
| | 100 | 12,3±2,26 | 9 | 17 | | |
| *Culiseta longiareolata* | Witness | 61,24±3,91 | 56 | 69 | $F^{3, 39}$=203 | .000 |
| | 10 | 43,1±1,97 | 39 | 46 | | |
| | 50 | 30,2±3,55 | 24 | 34 | | |
| | 100 | 15,9±3,28 | 10 | 20 | | |

The number of eggs laid to females witnesses little to reach 69 and 68 eggs successively at *Cs. longiareolata* and *Cx. pipiens*, Then this number was too weak which is conversely correlated to the dose used, and did not exceed 9 and 10 eggs in lots treated by the dose 100 mg / L. The analysis of the variance (ANOVA) for laid eggs indicated that there is a significant difference between the individual's witnesses and treaties. The Test Chi-Square (dose, species and number of the eggs laid) showed that it is not a significant difference ($\chi^2$ = 3,91; DLL=3; p=0,270).

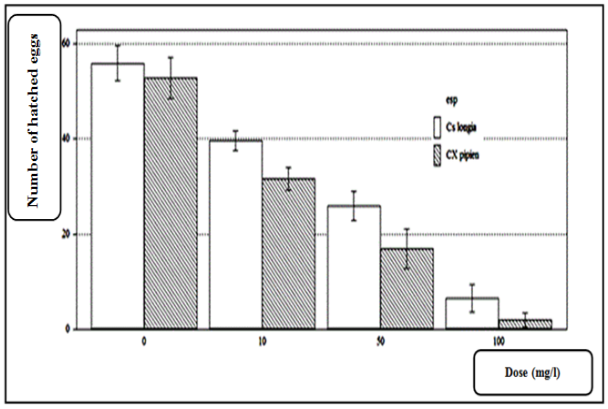


**Fig 1**: Number of eggs hatched of *Cx. pipiens* and *Cs. longiareolata*

The Test Chi-Square (dose, species and number of the hatched eggs) showed that it is not a significant difference ($\chi^2$ =30, 41; DLL=3; p=0,000) (fig 1). Like the results obtained and for all the doses used, we observed that the number of eggs produced by the individuals of *Cs. longiareolata* was higher than the number of eggs produced of *Cx. pipiens.*

### 3.2 Effect of *Citrullus colocynthis* on the sterility of treated Individus

Among the parameters of reproduction which we considered in this study, the sterility index is a good indicator of who can show the effect of our product of treatment on the reproductive potential. The both figures 2 and 3 present the acquired results.

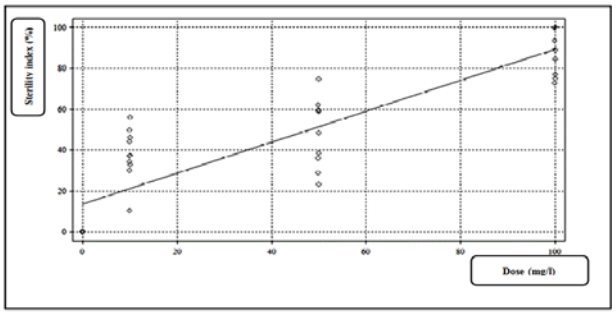


**Fig 2**: The sterility index of *Cx. pipiens*

The sterility index calculate was more raised at *Cx. pipiens* (58 %) than at *Cs. longieolata* (44, 95%) (F3, 79 = 12, 1; P=0,000). Besides, this sterility index was variable according to the used dose (F3, 39 = 96, 0; P=0,000), at the individuals of *Cx. pipiens*; it was of the order of 37, 83% for the dose 10 mg/l, and of the order of 48, 92% for the second dose of 50 mg/l and a 89, 2% report for the third dose of 100 mg/l (Fig. 2). Contrary, to *Cs. longiareolata*, this index was 31, 42% for the first dose, 34, 45% for the second dose and 75, 20% for the third dose (Fig. 3). Effectively, the correlation between the sterility index and the dose used was significant (r = 0.88, r=0.82) respectively at *Cx. pipiens* and *Cs. longiareolata*.

The sterility index was very high at *Culex pipiens* 90% of which it reached, contrary; it was of the order of 70% to *Culiseta longiareolata* (Fig 2, Fig 3). The analysis of the variance between the individuals treated with both species showed that it has no significant difference (P=0,189), then it there was significant between the treated and untreated individuals (p=0,000).

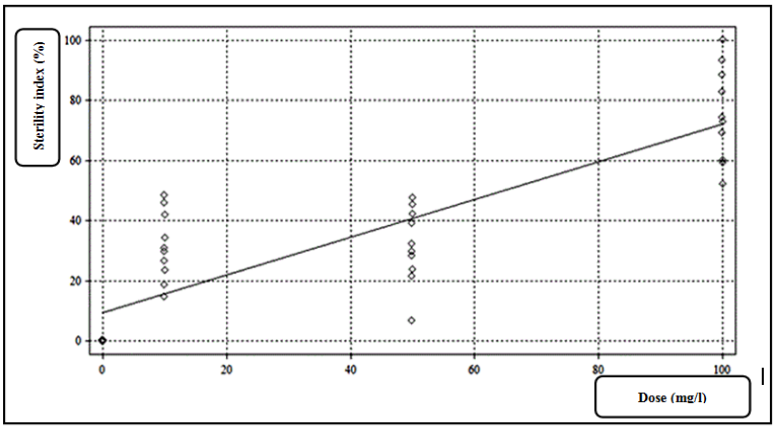


**Fig 3**: The sterilty index of *Cs. longiareolata*

## 4. Discussion

The two species of *Culicidae* included in this study are very wide-spread in Algeria and especially in the Saharan region [6]. The toxicity of the aqueous extract of fruits of *Citrullus colocynthis* was demonstrated in the toxic attempts applied for both species [20]. The rate of the fertility to the populations - insects for example allows measuring its tendency to be increased or to be naturally decreased. It is necessary to perturb this rate to fight against these natural enemies [21]. The effect of insecticides of natural origin; the extracts of plants for example on the reproductive parameters of some family of insects generally was realized (Crambidae, Noctuidae, Pyralidae and Tortricidae) [22-23-24], Or notably on mosquitoes showed that the differences were significant between at the witnesses and treaties individuals [24-25].

The results obtained in this study show that the parameter's reproduction studied are affected in adult females of emerged larvae treated on ($L^4$) stage. Disruption of reproductive parameters caused by the extract used can be explained either by sexual confusion that disruption of the hormonal system responsible for reproduction on one side [26], binding to specific nuclear receptors of the natural molting hormone (20 M) [27-28], or by decreased metabolite levels which may affect the maturation of eggs and the hatching percentage as the most larvae developed normally inside the eggshell but they have not hatched, because of low larval cuticle that has no mechanical force to cross the eggshell [29].

The treatment of newly laid eggs of *Cx. pipiens* reduced hatchability. The same effect is recorded when using inhibitors of chitin synthesis in respect of eggs of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* [30].

The treatment of larvae of the both species showed a reduction in the reproductive potential in females, through reducing the number of eggs laid between the treatment and control groups batches and with increasing doses, this reflects a reduction in the hatching rate (E%) eggs in both species, and the rate of fertility (F%).

Treating individuals has increased the sterility gradually with increasing the dose used and the exposure time. A multitude of works that contribute to strengthening research, including the lowering of fertility or increased sterility in insects in general [16], and specifically in mosquitoes which use radiation (radiation X, Gamma, Ultraviolet.) [31-32-13-14].

## 5. Conclusion

All information recorded in this study obviously deserve to be reinforced by further studies on this context, in particular it is important to further research on other plants that have toxic effects on reducing reproductive performance insects including species at risk of transmitting or causing a nuisance intense. Other studies such as the mode of action and synergy with the use of solvents to extract the active ingredients of this molecule and its effects on reproduction parameters in species of *Culicidae* are needed.

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